CLAIMS

1. Method for preparing a specific recombinant protein, said method being carried out by overexpression of the gene encoding for this specific protein in a monokaryotic strain of filamentous fungi of the species *Pycnoporus* of the basidiomycete group, and comprises:

- a stage of culturing the abovementioned monokaryotic strain of *Pycnoporus*, said strain being transformed using an expression vector containing the gene encoding for the specific recombinant protein, the expression of which is placed under the control of a promoter corresponding to an endogenous promoter of the abovementioned fungi, or of a different promoter (also designated exogenous promoter), said promoter being constitutive or inducible,
- if appropriate a stage of induction of the abovementioned promoter, when the latter is inducible,
- the recovery, and, if appropriate, the purification of the specific recombinant protein, produced in the culture medium.

2. Method according to claim 1, characterized in that the monokaryotic strain of *Pycnoporus* used for the overexpression of the gene encoding for the specific recombinant protein, is as obtained by culturing the original dikaryotic strain at 30°C in the dark for 15 days, followed by a stage of exposure to daylight for 2 to 3 weeks at ambient temperature until the formation of fruiting organs corresponding to differentiated hyphas called basidia, within which karyogamy then takes place, followed by meiosis which leads to the formation of four sexual spores, or genetically different haploid basidiospores, which, after germination, produces a monokaryotic mycelium.

3. Method according to claim 1 or 2, characterized in that the monokaryotic strain of *Pycnoporus* used is a strain of *Pycnoporus cinnabarinus*.

4. Method according to one of the claims 1 to 3, characterized in that the specific recombinant proteins overexpressed correspond to endogenous proteins of *Pycnoporus*, or to exogenous proteins, in particular exogenous proteins corresponding to endogenous proteins of basidiomycetes other than *Pycnoporus*, such as the basidiomycete enzymes

involved in plant biotransformations, or corresponding to endogenous proteins of strains of *Pycnoporus* different from the strain of *Pycnoporus* used for the production of said proteins.

5. Method according to one of the claims 1 to 4, characterized in that the specific recombinant proteins correspond:

- to the following endogenous proteins of *Pycnoporus*:

  * the metalloenzymes, such as laccase, or tyrosinase,

  * or cellobiose dehydrogenase, xylanase, β-glycosidase, invertase, or α-amylase,

- to the exogenous proteins chosen from the following:

  * the tyrosinases of strains of *Pycnoporus* different from the strain of *Pycnoporus* used for the production of said proteins, such as the tyrosinase of *Pycnoporus sanguineus* when the strain of *Pycnoporus* used for the production of this tyrosinase is different from *Pycnoporus sanguineus*,

  * the laccases of basidiomycetes other than *Pycnoporus*, such as the laccase of *halocyphina villosa* (halophilic basidiomycete),

  * the cinnamoyl esterases A and B of *Aspergillus niger*.

6. Method according to one of the claims 1 to 5, for preparing specific recombinant proteins corresponding to the endogenous proteins of *Pycnoporus*, characterized in that the monokaryotic strain of *Pycnoporus* used is deficient in the gene encoding for the endogenous protein to which the specific recombinant protein corresponds.

7. Method according to one of the claims 1 to 6, for preparing specific recombinant proteins corresponding to the endogenous proteins of *Pycnoporus*, characterized in that the monokaryotic strain of *Pycnoporus* used is transformed using an expression vector containing the gene encoding for the specific recombinant protein labelled in particular by a histidine label.

8. Method according to one of the claims 1 to 7, for preparing recombinant laccases corresponding to the endogenous laccases of *Pycnoporus*, characterized in that it comprises:

- a stage of culturing a monokaryotic strain of *Pycnoporus*, if appropriate deficient in the gene encoding for the endogenous laccase of *Pycnoporus*, transformed using an expression vector containing the gene encoding for a laccase of *Pycnoporus*, if appropriate labelled, and the expression of which is placed under the control of a promoter corresponding to the endogenous promoter of this laccase,

- a stage of induction of the abovementioned promoter, in particular by adding ethanol, or agricultural by-products containing lignocellulose such as wheat straw, corn bran and beet pulp, or compounds with an aromatic ring such as 2,5-xylidine, veratrylic acid, guaicol, veratrylic alcohol, syringaldazine, ferulic acid, caffeic acid and the lignosulphonates,

- the recovery, and, if appropriate, the purification of the recombinant laccase, if appropriate labelled, corresponding to the abovementioned endogenous laccase of *Pycnoporus* produced in the culture medium.

9. Method according to claim 8, for preparing the recombinant laccase corresponding to the endogenous laccase of *Pycnoporus cinnabarinus* represented by SEQ ID NO: 2, characterized in that it comprises:

- a stage of culturing a monokaryotic strain of *Pycnoporus cinnabarinus*, if appropriate deficient in the gene encoding for the endogenous laccase of *Pycnoporus cinnabarinus*, transformed using an expression vector containing the nucleotide sequence SEQ ID NO: 1 encoding for the recombinant laccase represented by SEQ ID NO: 2, if appropriate labelled, and the expression of which is placed under the control of the *pLac* promoter corresponding to the endogenous promoter of the abovementioned laccase, the sequence of said *pLac* promoter being represented by SEQ ID NO: 3,

- a stage of induction by ethanol of the abovementioned *pLac* promoter,

- the recovery, and, if appropriate, the purification of the recombinant laccase, if appropriate labelled, represented by SEQ ID NO: 2 produced in the culture medium.

10. Method for preparing recombinant laccases corresponding to the endogenous laccases of *Pycnoporus* according to one of claims 1 to 7, characterized in that it comprises:

- a stage of culturing a monokaryotic strain of *Pycnoporus,* if appropriate deficient in the gene encoding for the endogenous laccase of *Pycnoporus*, transformed using an expression vector containing the gene encoding for a laccase of *Pycnoporus*, if

appropriate labelled, the expression of which is placed under the control of an exogenous promoter chosen from:

* the *gpd* promoter of the expression of the gene encoding for the glyceraldehyde 3-phosphate dehydrogenase of *Schizophyllum commune*, the nucleotide sequence of which is represented by SEQ ID NO: 4,

* or the *sc3* promoter of the expression of the gene encoding for the hydrophobin of *Schizophyllum commune*, the nucleotide sequence of which is represented by SEQ ID NO: 5,

- the recovery, and, if appropriate, the purification of the recombinant laccase, if appropriate labelled, corresponding to the endogenous laccase of *Pycnoporus* produced in the culture medium.

11. Method according to claim 10, for preparing the recombinant laccase corresponding to the endogenous laccase of *Pycnoporus cinnabarinus* represented by SEQ ID NO: 2, characterized in that it comprises:

- a stage of culturing a monokaryotic strain of *Pycnoporus cinnabarinus*, if appropriate deficient in the gene encoding for the endogenous laccase of Pycnoporus, transformed using an expression vector containing the nucleotide sequence SEQ ID NO: 1 encoding for the recombinant laccase represented by SEQ ID NO: 2, if appropriate labelled, and the expression of which is placed under the control of the exogenous gpd or sc3 promoter,

- the recovery, and, if appropriate, the purification of the recombinant laccase, if appropriate labelled, represented by SEQ ID NO: 2 produced in the culture medium.

12. Method according to one of the claims 1 to 5, for preparing recombinant tyrosinase corresponding to the tyrosinase of *Pycnoporus sanguineus* represented by SEQ ID NO: 16, characterized in that it comprises:

- a stage of culturing a monokaryotic strain of *Pycnoporus cinnabarinus* transformed using an expression vector containing the nucleotide sequence SEQ ID NO: 15 encoding for the recombinant tyrosinase represented by SEQ ID NO: 16, if appropriate labelled, the sequence SEQ ID NO: 15 being advantageously preceded by the nucleotide sequence delimited by the nucleotides situated at positions 128 and 190 of SEQ ID NO: 1 encoding for the peptide signal of *Pycnoporus cinnabarinus* delimited by the first 21 amino acids of SEQ ID NO: 2, and the expression of which is placed

under the control of the *pLac* promoter corresponding to the endogenous promoter of the laccase of *Pycnoporus cinnabarinus*, the sequence of said *pLac* promoter being represented by SEQ ID NO: 3,

- a stage of induction by ethanol of the abovementioned *pLac* promoter,

- the recovery, and, if appropriate, the purification of the recombinant tyrosinase, if appropriate labelled, represented by SEQ ID NO: 16 produced in the culture medium.

13. Method according to one of the claims 1 to 5, for preparing recombinant laccase corresponding to the laccase of *halocyphina villosa* represented in Figure 12 (SEQ ID NO: 18), characterized in that it comprises:

- a stage of culturing a monokaryotic strain of *Pycnoporus cinnabarinus*, if appropriate deficient in the gene encoding for the endogenous laccase of *Pycnoporus cinnabarinus*, transformed using an expression vector containing the nucleotide sequence represented in Figure 12 (SEQ ID NO: 17) encoding for the recombinant laccase represented by SEQ ID NO: 18, if appropriate labelled, and the expression of which is placed under the control of the *pLac* promoter corresponding to the endogenous promoter of the laccase of *Pycnoporus cinnabarinus*, the sequence of said *pLac* promoter being represented by SEQ ID NO: 3,

- a stage of induction by ethanol of the abovementioned *pLac* promoter,

- the recovery, and, if appropriate, the purification of the recombinant laccase, if appropriate labelled, represented by SEQ ID NO: 18 produced in the culture medium.

14. Nucleotide sequence encoding for the *pLac* promoter of the endogenous laccase of *Pycnoporus cinnabarinus*, and corresponding to the sequence SEQ ID NO: 3, or any sequence derived from this promoter by substitution, addition or suppression of one or more nucleotides and retaining the property of being a promoter of the expression of sequences.

15. Expression vector characterized in that it comprises the sequence SEQ ID NO: 3 of the promoter *pLac* according to claim 14.

16. Expression vector according to claim 15, characterized in that it comprises a gene encoding for a specific recombinant protein, and the expression of which is placed under the control of the *pLac* promoter according to claim 14.

17. Expression vector according to claim 15 or 16, characterized in that the specific recombinant protein is a protein corresponding:

- to the following endogenous proteins of *Pycnoporus*:

  * the metalloenzymes, such as laccase, or tyrosinase,

  * or cellobiose dehydrogenase, xylanase, β-glycosidase, invertase, or α-amylase,

- to the exogenous proteins chosen from the following:

  * the tyrosinases of strains of *Pycnoporus* different from the strain of *Pycnoporus* used for the production of said proteins, such as the tyrosinase of *Pycnoporus sanguineus* when the strain of *Pycnoporus* used for the production of this tyrosinase is different from *Pycnoporus sanguineus*,

  * the laccases of basidiomycetes other than *Pycnoporus*, such as the laccase of *halocyphina villosa* (halophilic basidiomycete),

  * the cinnamoyl esterases A and B of *Aspergillus niger*.

18. Host cell transformed using an expression vector according to one of claims 15 to 17.

19. Host cell according to claim 18, corresponding to monokaryotic cells of strains of *Pycnoporus,* such as strains of *Pycnoporus cinnabarinus*.

20. Use of expression vectors according to one of claims 15 to 17, or of host cells according to claim 18 or 19, for the implementation of a method for overproducing a specific recombinant protein according to one of claims 1 to 13.